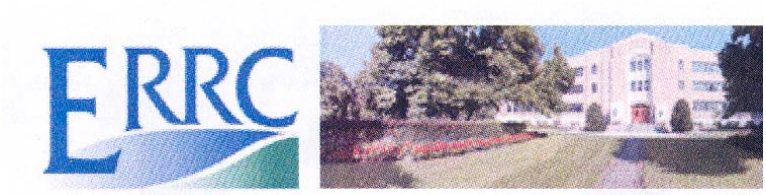

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Title: Radiation Resistance of Listeria monocytogenes in the Presence or Absence of Sodium Erythorbate

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Radiation Resistance of *Listeria monocytogenes* in the Presence or Absence of Sodium Erythorbate

C.H. SOMMERS, A.P. HANDEL, AND B.A. NIEMIRA

ABSTRACT: ***Listeria monocytogenes* is a common contaminant of ready-to-eat meat products that can be eliminated by low dose ionizing radiation. Sodium erythorbate (SE), an antioxidant, is commonly included in cured meat emulsions or applied to the surfaces of cured meats as a solution prior to packaging. The radiation resistance (D_γ) of *L. monocytogenes* increased when suspended in SE solutions of 0.1% and greater. However, no differences in D_γ, which ranged from 0.67 kGy to 0.70 kGy, were observed when *L. monocytogenes* was inoculated onto cooked cured meat products (frankfurters or bologna slices) which contained no SE, 0.05% SE in the emulsion, or a 10% SE solution applied to the product surface. Surface antioxidant power of the cured meats did not reach a level sufficient to protect *L. monocytogenes* against the lethal effects of ionizing radiation. Therefore, the industrial practice of applying SE to the surfaces of cured meat products would not compromise the efficacy of irradiation as an antimicrobial process.**

Keywords: food irradiation, antioxidant, protection

Introduction

LISTERIA MONOCYTOGENES IS A FOOD-BORNE PATHOGEN CAPABLE OF growth at refrigerated temperatures and in high salt environments (Smith 1996). It is a frequent post-process contaminant of ready-to-eat meat products. Approximately 2.5% of ready-to-eat meat products tested by the USDA's Food Safety Inspection Service in 1998 tested positive for *L. monocytogenes* (Nickelson and Schmidt 1999). A number of food-borne illness outbreaks and recalls have been attributed to *L. monocytogenes* (Anonymous 1998; Barnes and others 1989). Because of the high mortality rate associated with listeriosis, approximately 20% in susceptible populations, *L. monocytogenes* is given zero tolerance in ready-to-eat meat products (Mead and others 1999; USDA 1989).

Ionizing radiation can eliminate *L. monocytogenes* from cured meat products (Sommers and Thayer 2000). Antioxidants, including sodium erythorbate, are added to the emulsion of cured meat products or may be applied to the surfaces of cured meats immediately prior to packaging (Anonymous 1995). The radiolysis of water produces reactive oxygen species that are lethal to microorganisms (Ward 1991). The presence of antioxidants increases the radiation resistance of food-borne pathogens by reacting with the radiolytic products of water (Kim and Thayer 1995; Sharma and others 2000).

This work addressed the following questions: Does the antioxidant sodium erthythorbate protect *L. monocytogenes* against the effects of ionizing radiation in solution? Does the addition of sodium erythorbate to cured meat emulsion or its application to cured meat product surfaces affect the radiation resistance of *L. monocytogenes*?

Materials and Methods

Strains

Four *L. monocytogenes* isolates (7644, 15313, 43256, 49594) were obtained from the American Type Culture Collection (Manassas, Va., U.S.A.). The strains were propagated on Tryptic Soy Agar (Difco Laboratories, Detroit, Mich., U.S.A.) at 37 °C and maintained at 0 to 2 °C until ready for use. Strain identity was confirmed by gram stain followed by analysis with Gram Positive Identification (GPI) cards using the Vitek Automicrobic System (bioMerieux Vitek, Inc., Hazelwood, Mo., U.S.A.).

Bacterial cultures

Each *L. monocytogenes* strain was cultured independently in 100 mL Tryptic Soy Broth (Difco Laboratories, Detroit, Mich., U.S.A.) in a baffled 500 mL Erlenmeyer culture flask at 37 °C (150 rpm) for 18 h. The cultures were then combined and the mixture pelleted by centrifugation (1725 × g for 30 min). The *L. monocytogenes* cells were then concentrated ten-fold by re-suspension in 40 mL of Butterfield's Phosphate Buffer (BPB) (Applied Research Institute, Newtown, Conn., U.S.A.).

L. monocytogenes sodium erythorbate solution survival curves

Filter sterilized (0.2 μm) 20% weight/volume sodium erythorbate solution (pH 6.0) (Spectrum, Inc., New Brunswick, N.J., U.S.A.) was freshly prepared and diluted in sterile distilled water prior to each experiment. Concentrated *L. monocytogenes* cocktail (2.5 mL) was diluted with an equal volume of appropriately diluted sodium erythorbate to final concentrations of 10%, 1%, 0.1%, 0.01%, 0.001%, and 0%. Following serial dilution in BPB, samples were irradiated (sample temperature of 4 °C) within 1 h of preparation, and pour plated using TSA (Tryptic Soy Agar) (Sommers and Thayer 2000).

Cured meat manufacture

To maintain control over formulation and manufacturing practices, all frankfurters and bologna were produced in house. Standard procedures and formulations were used (Rust 1976; Ockerman 1989). Ground beef (85 trim) was emulsified in a Hobart Model HCM40 Cutter-Mixer (Hobart, Colo., Marietta, Ga., U.S.A.). Cure ingredients and additives (w/w per kg meat) included 3% sodium chloride, 3% dextrose, 0.5% sodium tripolyphosphate, 0.05% sodium erythorbate, 200 mg/kg sodium ni-

trite, and 20% deionized water. Spices and smoke were not used in order to limit the number of experimental variables. For frankfurters the emulsion was stuffed into 22 mm clear cellulose casings (Dewied Int., Sante Fe, N.Mex., U.S.A.), with an average frankfurter length of 10 cm. For manufacture of ring bologna, the emulsion was stuffed into 44.5 mm fibrous casings. Both the frankfurters and bologna were cooked in a Koch Model KL-50 Smokehouse (Koch Inc., Kansas City, Mo., U.S.A.) to an internal product temperature of 73 °C. The dry bulb setting was 90 °C, and wet bulb setting was 63 °C, for a relative humidity of approximately 47%.

After the internal temperature was reached, the meats were cooled to an internal temperature of 33 °C using a cold water shower. The casings were removed and the meats placed in Nr 400 Stomacher bags (Tekmar, Inc., Cincinnati, Ohio, U.S.A.), vacuum packed to 0.23 mmHg using a Multi-Vac A300 Vacuum Packager (Multi-Vac, Kansas City, Mo., U.S.A.). They were then overpacked in Mil-B-131-H Foil bags (Bell Fibre Products Corp., Columbus, Ga., U.S.A.) and stored at 0 to 2 °C until ready for use.

Background microflora was monitored by pour plate assay (see above) over the course of the study and contributed less than 1 $\log_{10}$ CFU/cm^2 to the inoculated meat product at the lowest dilution used. Experiments were completed within 10 d of meat manufacture.

L. monocytogenes cured meat survival curves

Individual frankfurters or bologna slices (sliced manually to approximately 4 mm thick) were dipped in sterile water or 10 percent sodium erythorbate solution, and placed in Nr 400 Stomacher bags (Tekmar, Inc., Cincinnati, Ohio, U.S.A.). The meat samples were then surface-inoculated with 200 µL (about 1.0×10^9 CFU) of concentrated *L.°monocytogenes* cocktail, vacuum-packed to 0.23 mmHg using a Multi-Vac Model A300 packager (Multi-Vac, Kansas City, Mo., U.S.A.). They were then overpacked in Mil-B-131-H Foil bags (Bell Fibre Products Corp., Columbus, Ga., U.S.A.) and stored at 0 to 2 °C for about 30 minutes until irradiation. The inoculated meat was irradiated (sample temperature 4 °C), diluted in BPB, and pour plated using TSA medium (Sommers and Thayer 2000).

Irradiator

A self-contained ^{137}Cs gamma radiation source (Lockheed Georgia Co., Lockheed Georgia Co., Marietta, Ga., U.S.A.) was used for all exposures. The radiation source consisted of 23 individually sealed source pencils placed in an annular array. The 22.9 cm × 63.5 cm cylindrical sample chamber was located central to the array when placed in the operating position. The inoculated vacuum-packed frankfurters were placed vertically in the sample chamber to insure uniformity of dose.

The dose rate provided by the irradiator was 0.101 kGy/min. The temperature during irradiation process was maintained at 4.0 ± 1.0) °C by the gas phase of a liquid nitrogen source which was introduced directly into the top of the sample chamber. The temperature was monitored during the entire irradiation process using 2 thermocouples placed 1 to 2 cm adjacent to the samples. The dose delivered was verified by use of 5 mm alanine pellet dosimeters, which were then measured using a Brucker EMS 104 EPR Analyzer (Brucker, Inc., Billerieca, Mass., U.S.A.).

D_γ-Values

D_γ is defined as the radiation dose required to produce a 90% reduction in viable organisms. The average CFU/plate of an irradiated sample (N) was divided by the average CFU/plate of the untreated control (N_o) to produce a survivor ratio (N/No). D_γ was determined by calculating the reciprocal of the slope provided by the (N/No) ratios. Each experiment was conducted independently 3 times. Doses of 0.5, 1.0, 1.5, 2.0, and 2.5 kGy were used for determination of D_γ for the sodium erythorbate solution experiments. Doses of 0.4, 0.8, 1.2, 1.6, 2.0, and 2.4 kGy were used for D_γ determination in the cured meat product experiments.

Table 1—Radiation resistance of *L. monocytogenes* in sodium erythorbate solutions

%Sodium Erythorbate (weight/volume)	µmoles sodium erythorbate (per 5.0 ml sample)	R^2	Dg (kGy)*
0.000% (0.00 µM)	0	0.94	0.35 (± 0.02)
0.001% (463 µM)	0.231	0.86	0.32 (± 0.02)
0.010% (4.63 µM)	2.310	0.84	0.35 (± 0.03)
0.100% (46.3 µM)	23.10	0.91	0.60 (± 0.04)
1.000% (463 µM)	231.0	0.94	0.70 (± 0.01)
10.00% (0.46 M)	2310	0.81	0.88 (± 0.10)

*Data shown are the result of 3 independent experiments. Standard error is shown in parenthesis.

FRAP assay

Total antioxidant power of cured meat was measured directly by the ferric reducing/antioxidant power (FRAP) assay (Benzie and Strain 1999). In the assay, antioxidants present reduce ferric tripyridyltriazine at low pH to the ferrous form, which has an intense blue color. Absorbance was measured at 593 nm and concentration calculated against a standard curve of sodium erythorbate (0 to 500 µM).

Bologna slices (44.5mm D × 3 to 4 mm thick, 6.65 ± 0.88 g), with and without the sodium erythorbate treatments previously described, were vacuum packaged (n = 6) in Nr 400 Stomacher bags. After packaging, samples were stored at 4 °C until analyzed. Upon cutting open a sample, 40 mL of distilled water was added and the slice shaken with the water for 30 s. The rinse water was added to a 100 mL volumetric flask. A second 40 mL of water was added to the bag, the sample was shaken another 30 s, and the rinses combined in the flask. The flask was filled to the mark with distilled water. Results of the FRAP assay are expressed in terms of mmoles of sodium erythorbate per cm^2 of bologna surface area.

Statistical analysis

Statistical analysis was completed using the statistical analysis package of Microsoft Excel (Microsoft Corp., Redmond, Wash., U.S.A.) and SAS Version 6.12 (SAS Institute, Cary, N.C., U.S.A.). Comparison of regressions was performed using ANOVA and ANCOVA. Sigma Plot Version 5.0 (SPSS, Inc., Chicago, Ill., U.S.A.) was used for determination of a predictive equation and graphic presentation of the data.

Results and Discussion

Results

The radiation resistance of *L. monocytogenes* in the presence of sodium erythorbate solutions was determined. The viability of *L. monocytogenes* prior to irradiation was not affected by suspension in sodium erythorbate solution as determined by ANOVA ($n = 3, \alpha = 0.05$). The radiation resistance of *L. monocytogenes* suspended in BPB increased with increasing concentration of so-

dium erythorbate (Table 1). *L. monocytogenes* D_γ became significantly different from the 0% sodium erythorbate control between 0.01% (2.3 μmoles/tube) and 0.1% (23 μmoles/tube) sodium erythorbate, as determined by both ANOVA and ANCOVA ($n = 3, \alpha = 0.05$).

A 3-D mesh plot showing radiation-induced log reduction data (N/No) in the presence of sodium erythorbate solutions is shown in Figure 1. The data follow a parabolic distribution. The best-fit predictive equation ($R^2 = 0.84$) for a 3-D regression is $N/No = (-2.69 \times D) + (1.84 \times E) + (0.14 \times D^2) - (0.16 \times E) - 0.742$, where D is the radiation dose (kGy) and E is the percentage of the sodium erythorbate solution.

The radiation resistance of *L. monocytogenes* inoculated onto frankfurters with differing sodium erythorbate concentrations and application methods is shown in Figure 2. The viability of *L. monocytogenes* applied to the surfaces of frankfurters was not affected by sodium erythorbate as part of the emulsion or applied to the product surface (ANOVA, $n = 3, \alpha = 0.05$). D_γ for *L. monocytogenes* was 0.67 (± 0.01) kGy when inoculated onto beef franks without sodium erythorbate (Figure 2A), 0.67 (± 0.01) on franks with 0.05% sodium erythorbate in the emulsion (Figure 2B), and 0.67 (± 0.01) kGy when inoculated onto franks dipped in 10% sodium erythorbate solution (Figure 2C). Differences in D_γ for *L. monocytogenes* inoculated onto the 3 frankfurter types were non-significant as determined by both ANOVA and ANCOVA ($n = 3, \alpha = 0.05$).

The radiation resistance of *L. monocytogenes* inoculated onto bologna slices with differing sodium erythorbate concentrations and application methods is shown in Figure 3. The viability of *L. monocytogenes* on bologna slices prior to irradiation was not affected by sodium erythorbate application method as determined by ANOVA ($n = 3, \alpha = 0.05$). D_γ for *L. monocytogenes* was

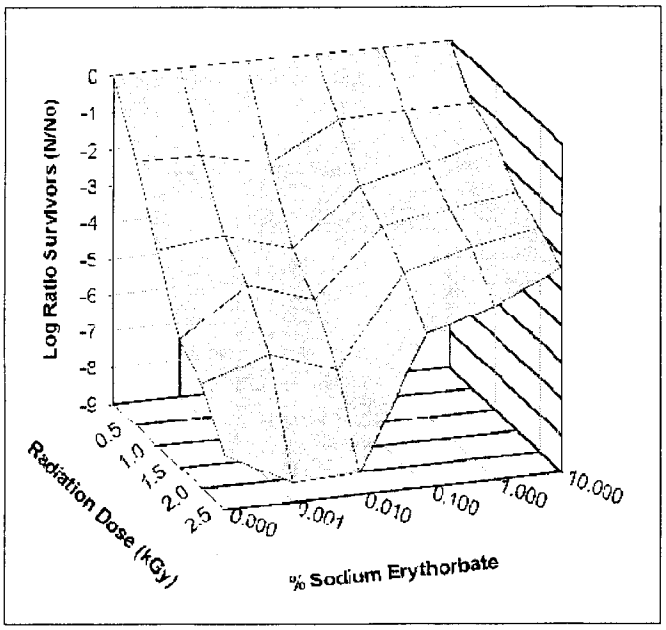

Figure 1—Radiation resistance of *L. monocytogenes* suspended in sodium erythorbate solutions The 3D mesh shown is the result of 3 independent experiments. The best-fit predictive equation ($R^2 = 0.84$) for a 3-D regression based on a parabolic model is N/No = (-2.69 x D) + (1.84 x E) + (0.14 x D^2) – (0.16 x E) – 0.742, where D is the radiation dose (kGy) and E is the percentage of the sodium erythorbate solution.

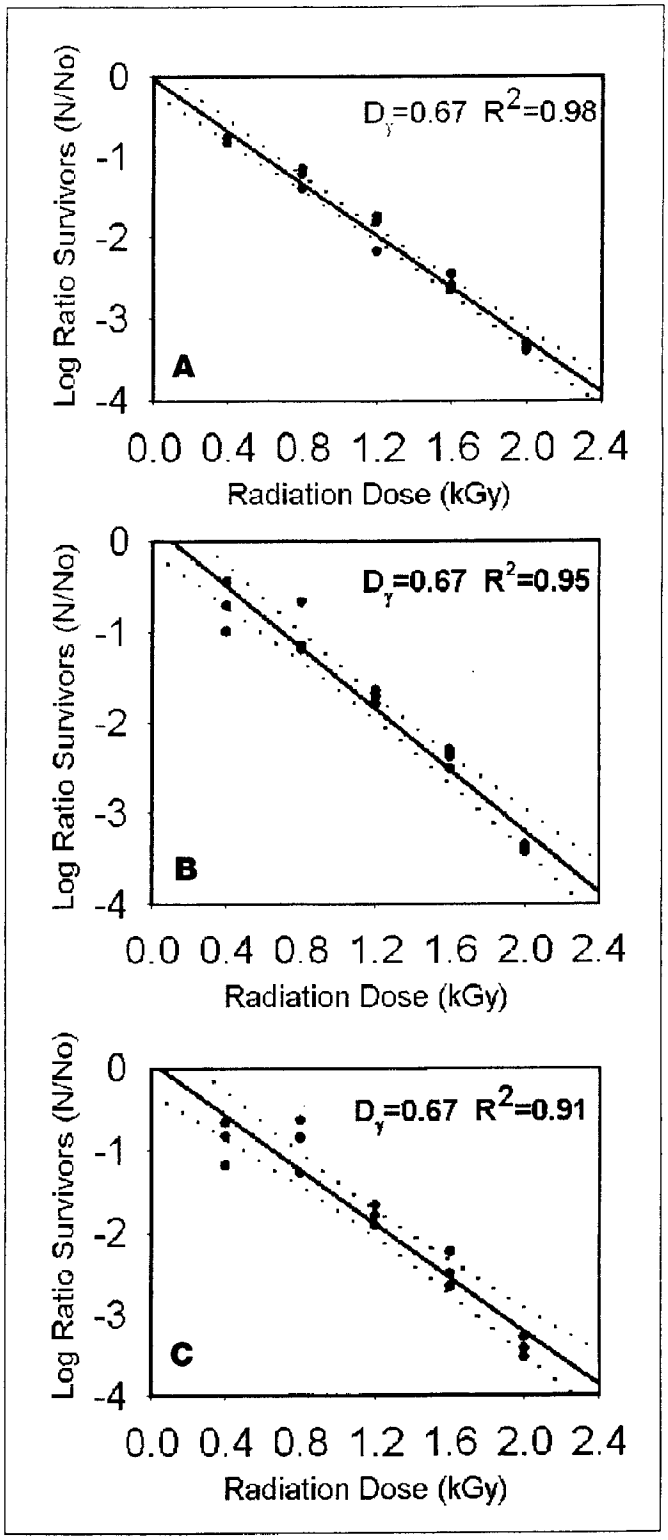

Figure 2—Radiation resistance of *L. monocytogenes* surface-inoculated onto beef frankfurters containing: (A) 0.05% sodium erythorbate in the emulsion with sterile distilled water applied to the frankfurter surface. (B) No sodium erythorbate in the frankfurter emulsion and 10% sodium erythorbate solution applied to the frankfurter surface. (C) No sodium erythorbate in the frankfurter emulsion with sterile distilled water applied to the frankfurter surface. Each experiment was conducted independently 3 times. Confidence intervals (95%) are shown as the dashed lines.

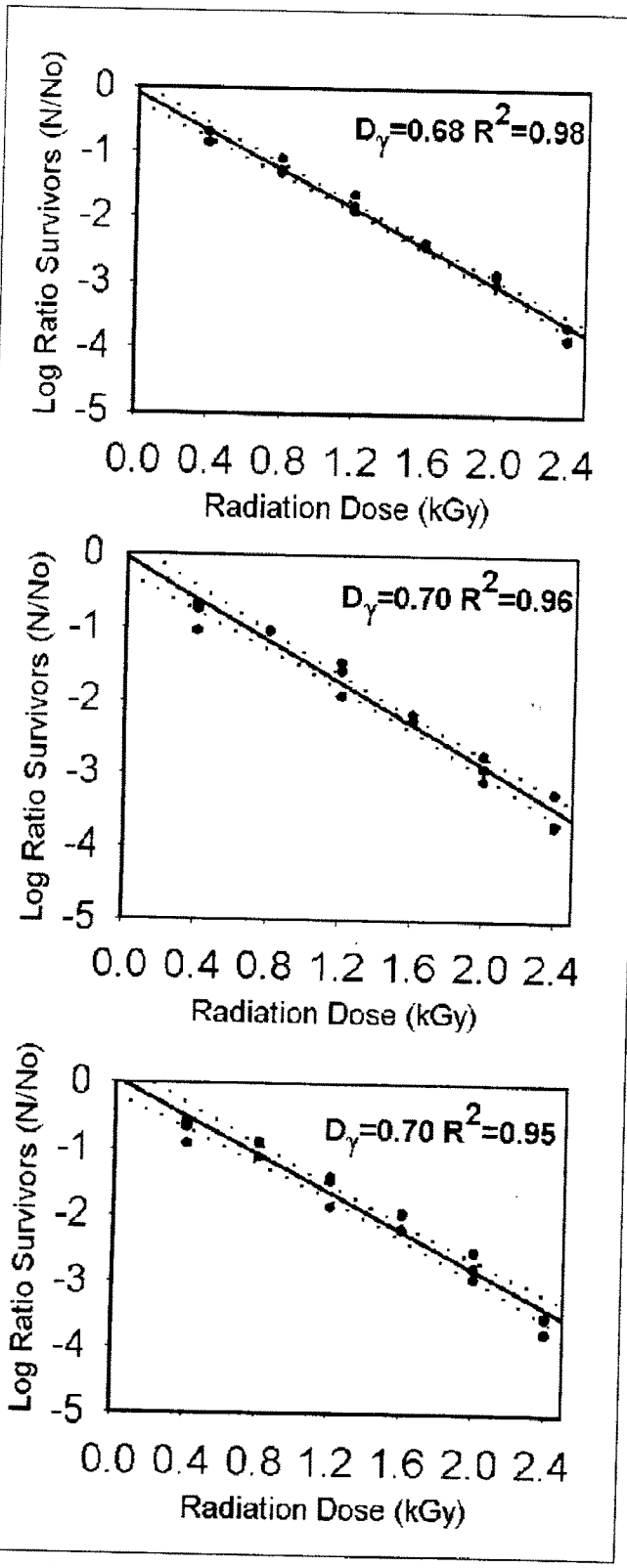

Figure 3—Radiation resistance of *L. monocytogenes* surface-inoculated onto beef bologna slices containing: (A) 0.05% sodium erythorbate in the emulsion with sterile distilled water applied to the bologna slice surface. (B) No sodium erythorbate in the frankfurter emulsion and 10% sodium erythorbate solution applied to the bologna slice surface. (C) No sodium erythorbate in the frankfurter emulsion with sterile distilled water applied to the bologna slice surface. Each experiment was conducted independently 3 times. Confidence intervals (95%) are shown as the dashed lines.

0.70 (± 0.02) kGy when surface-inoculated onto bologna slices without sodium erythorbate (Figure 3A), 0.70 (±0.02) kGy on bologna with 0.05% sodium erythorbate in the emulsion (Figure 3B), and 0.68 (± 0.01) kGy when inoculated onto bologna dipped in 10% sodium erythorbate solution (Figure 3C). Differences in D_γ of *L. monocytogenes* on the 3 bologna types were not significant as determined by both ANOVA and ANCOV ($n = 3, \alpha = 0.05$).

FRAP assays performed on bologna slices without sodium erythorbate yielded a FRAP erythorbate equivalance values of 0.529 μmoles per slice (0.016 μmoles per cm^2 surface area). Bologna slices with 0.05% sodium erythorbate in the emulsion had a FRAP erythorbate equivalance of 3.76 μmoles per slice (0.11 μmoles per cm^2 surface area). Bologna slices dipped in 10% sodium erythorbate solution had a FRAP erythorbate equivalance of 39.6 μmoles per slice (1.91 μmoles per cm^2 surface area).

Discussion

The D_γ of *L. monocytogenes* inoculated onto cured meat products, 0.67 kGy to 0.70 kGy, are consistent with those obtained in other studies. D_γ for *L. monocytogenes* surface-inoculated onto commercially available frankfurters ranged from 0.49 kGy to 0.71 kGy (Sommers and Thayer 2000). D_γ's for *L. monocytogenes* of 0.58 to 0.69 kGy were obtained following suspension in raw and cooked turkey (Thayer and others 1998). *L. monocytogenes* D_γ's of 0.59 and 0.68 kGy were obtained using uncooked and cooked chicken breast meat as the suspending media (Shamsuzzaman and others 1992).

Ionizing radiation kills microorganisms by direct damage to genetic material or by the indirect effects of reactive oxygen species, produced by the radiolysis of water, on cell membranes and chromosomes (Ward 1991). However, the ability of ionizing radiation to eliminate food-borne pathogens can be attenuated by the presence of antioxidants. The antioxidants formate and polyethylene glycol protected *Salmonella typhimurium* against the effects of ionizing radiation when suspended in Butterfield's Phosphate Buffer (Kim and Thayer 1995). Spice extract solutions increase the radiation resistance of *Escherichia coli, Bacillus megaterium,* and *Bacillus pumilus* (Sharma and others 2000). When *L. monocytogenes* was suspended in sodium erythorbate solution, a dose dependent increase in the radiation resistance of *L. monocytogenes*, from 0.35 kGy at 0% sodium erythorbate, to 0.88 kGy in 10% sodium erythorbate, was observed.

Sodium erythorbate (D-sodium isoascorbate) is a non-vitamin stereoisomer of L sodium ascorbate that is used in the manufacture of cured meat products (Anonymous 1995). It is especially useful for preservation of color in sliced luncheon meat products (Anonymous 1995). Proctor and others (1952) found that sodium erythorbate protects food products against ionizing radiation induced chemical changes. However, in this study, sodium erythorbate did not affect the radiation resistance of *L. monocytogenes* when incorporated into the emulsion of a cooked cured meat product, or when applied to the surface of cooked and cured meat products as a 10% solution immediately prior to packaging.

In solution, erythorbate can more readily interact with radicals produced by the radiolysis of water, thereby preventing the interaction of those radicals with suspended *L. monocytogenes*. Concentrations of sodium erythorbate as low as 0.1% (23.1 μmoles of antioxidant) increased the D_γ of *L. monocytogenes* inoculum in the solution studies. The FRAP determined antioxidant activity of cured meat containing 0.05% sodium erythorbate in the emulsion indicated the exposure of the inoculum was only

3.76 μmoles antioxidant (0.11 μmoles per cm^2). Surface antioxidant activity of bologna slices dipped in 10% sodium erythorbate solution exposed the L. *monocytogenes* inoculum to 39.6 μmoles (1.91 μmoles per cm^2) antioxidant.

The difference in the availability of antioxidant between a solution and a meat system should be noted. Sodium erythorbate solution applied to cured meat product surfaces would be absorbed into the meat as a result of vacuum packaging, limiting its diffusion, and therefore its ability to protect *L. monocytogenes* from radiation produced free radicals. Sodium erythorbate would assist in the reduction of the cured meat nitroso-myoglobin complex, which can be destabilized by ionizing radiation (Kamarei and others 1981). In addition to the scavenging of free radicals produced by the radiolysis of water, erythorbate could also serve as a free radical acceptor for radicals produced by the radiolytic products of glucose (Von Sonntag 1987).

Erythorbate could interact with radicals produced by the radiolysis of free amino acids and polypeptides in the cured meat matrix (Von Sonntag 1987). Interaction would also occur with free radicals produced by peroxidation of lipids in cured meat product (Kanat and others 1997).

Conclusions

SOLUTION STUDIES PROVIDE VALUABLE INSIGHT INTO THE INTERACtions between antioxidants and the radiolytic products produced in vitro as a result of irradiation. However, meat products and cured meat product formulations represent complex systems that may not yield the same results obtained in simple solution studies. Conclusions concerning the antimicrobial activity of natural and synthetic antioxidants must be based on actual product formulations and manufacturing processes currently used by the meat processing industry.

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